International Citrus Canker Research Workshop

Ft. Pierce, FL • June 20-22, 2000

Citrus Canker Research Workshop
Ft. Pierce, FL
June 20-22, 2000

Mission Statement: development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

DAY 0: PRE-MEETING PLANNING - MONDAY, 19 JUNE 2000

7:30 - 8:30 PM **Social Hour at Radisson Beach Resort Facilities: cash bar available**

8:30 - 9:30 PM **Workshop Banquet at Radisson Beach Resort Facilities:**
Prime rib entrée $25; Chicken picata $21 at workshop participants' expense
Limited to workshop participants and invited guests only

9:30 - 10:00 PM **Speakers' and Moderators' Organizational Meeting**

DAY 1: RESEARCH REPORTS – WHAT IS KNOWN - TUESDAY, 20 JUNE 2000

7:30 – 8:00 AM **Registration**

8:00 – 8:15 AM **Welcome/Introduction** — Tim Gottwald
Mission / Goals/ Objectives — Andy LaVigne
Housekeeping/Rules of the House — Laurene Levy, Wayne Dixon

8:15 – 8:45 AM **An Overview of Argentina's Citrus Canker Control Program with Applicable Costs for a Similar Program in Florida** - Ron Muraro, Fritz Roka, and Thomas Spreen

8:45 – 9:45 AM **Epidemiology:**
Estimating Spread of Citrus Canker in Urban Miami via Differential GPS – Tim Gottwald, Xiaoan Sun, Tim Riley, James Graham, and Gareth Hughes

Epidemiology of Citrus Canker in Brazil with and without Asian Citrus Leaf Miner – Armando Beramin-Filho, Lilian Amorim, Francisco Laranejeira, and Tim Gottwald

9:45 – 10:00 AM **Break refreshments provided by the Florida Citrus Industry**

10:00 – 12:00 PM **Survival and Control:**
Survival of Xanthomonas campestris pv. citir (Xcc) on Various Surfaces and Chemical Control of Asiatic Citrus Canker (ACC) - James Graham, Tim Gottwald, Tim Riley, Jaime Cubero, And D. Drouillard

Control Studies in Brazil – Rui Leite, Jr.

The workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and key representatives for the commodity groups in the citrus industry. Invited scientists are charged with developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The FCCTATF is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida

Steering Committee Co-chairman: Tim Gottwald, Laurene Levy, Wayne Dixon
Members: Richard Gaskalla, Andy LaVigne, Tom Jerkins, Dean Gabriel, Randy Niedz, Michael Hunt, Tim Schubert, Mike Carlton, James Graham, Arnold Tschanz

Mission Statement: development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

Citrus Bacterial Canker Disease: A Review on Research Developed on Réunion Island France - Olivier Pruvost, Christian Vernier, Annie Couteau, Bernard Boher, and Michel Nicole

Survival and Control Experiments in Argentina – Pete Timmer

12:00 – 1:00 PM **Lunch provided by the Florida Citrus Industry**

1:15 – 2:45 PM **Citrus Canker in Argentina – Control, Eradication, and Current Management –** Blanca Isabel (Nelly) Canteros

Methods for Detection and Characterization of *Xanthomonas axonopodis* pv. *citri* – Jaime Cubero, James Graham, and Tim Gottwald

Integrating the Scientific Perspective into Citrus Canker Eradication Efforts in Florida – Tim Schubert, Xiaoan Sun, and Wayne Dixon

2:45 – 3:00 PM **Break refreshments provided by the Florida Citrus Industry**

3:00 – 5:30 PM **Detection and Differentiation:**

Citrus Canker Detection and Diagnostics in Florida – Xiaoan Sun, Tim Schubert, Wayne Dixon, and Maria Peacock

Detection of an Unique Isolate of Citrus Canker Bacterium from Key Lime in Wellington and Lake Worth, Florida - Xiaoan Sun, Robert Stall, Jaime Cubero, Tim Gottwald, James Graham, Wayne Dixon, Tim Schubert, Maria Peacock, Bruce Sutton, Ellen Dickstein, and Paul Chaloux

Analysis of Fatty Acid Profiles of Xanthomonads Pathogenic to Citrus - Robert Stall, Ellen Dickstein, Jand effrey Jones

***Xanthomonas axonopodis* pv. *citri* Genome Project –** Jesus Ferro

Resistance:

Detection of *Xanthomonas axonopodis* pv. *citri* Bacteria and Differentiation of Strains - John Hartung

Canker Symptom Induction, Knowledge-based Resistance and Natural Resistance Potential of Citrus - Dean Gabriel

5:30 PM **Adjourn – Steering Committee Meeting**

The workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and key representatives for the commodity groups in the citrus industry. Invited scientists are charged with developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The FCCTATF is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida

Steering Committee Co-chairman: Tim Gottwald, Laurene Levy, Wayne Dixon
Members: Richard Gaskalla, Andy LaVigne, Tom Jerkins, Dean Gabriel, Randy Niedz, Michael Hunt, Tim Schubert, Mike Carlton, James Graham, Arnold Tschanz

Mission Statement: development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

DAY 2: NEW METHODOLOGIES AND APPROACHES - 21 JUNE 2000

Introductions to Moderators: Randy Niedz and Wayne Dixon

The moderators for each section will present a 10-20 minute overview of the topic followed by a general scientists-at-large discussion.

8:00 – 9:00 AM	**Remote Sensing and Spectral Analysis** – Chenghai Yang and Marcus Borengasser
9:00 – 9:30 AM	**Detection Technology** – Mike Syvanen and Michael Kosho
9:30 – 9:45 AM	**Break refreshments provided by the Florida Citrus Industry**
9:45 – 11:15 AM	**Citrus Breeding and Transformation** – Jose Chaparro, Vicente Ferbes, and Gloria Moore
11:15 – 12:00 PM	**Genomics, Resistance, and GMOs**– Dean Gabriel and John Hartung
12:00 – 1:15 PM	**Lunch provided by the Florida Citrus Industry**
1:15 – 2:00 PM	**Genomics, Resistance, GMOs continued**
2:00 – 3:15 PM	**Citrus Canker Epidemiology – Methodologies and Approaches -** Armando Bergamin-Filho and Gareth Hughes
3:15 – 3:30 PM	**Break refreshments provided by the Florida Citrus Industry**
3:30 – 4:30 PM	**Chemical Control Methods**—Anne Anderson and Pete Timmer
4:30 – 5:30 PM	**Biological and Cultural Control Methods**—Rui Leite and Tim Schubert
5:30 PM	**Adjourn** – Steering Committee Meeting

The workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and key representatives for the commodity groups in the citrus industry. Invited scientists are charged with developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The FCCTATF is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida

Steering Committee Co-chairman: Tim Gottwald, Laurene Levy, Wayne Dixon
Members: Richard Gaskalla, Andy LaVigne, Tom Jerkins, Dean Gabriel, Randy Niedz, Michael Hunt, Tim Schubert, Mike Carlton, James Graham, Arnold Tschanz

Mission Statement: development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

DAY 3: ESTABLISH RESEARCH PRIORITIES - THURSDAY, 22 JUNE 2000

8:00 – 10:00 AM	**Discussion and Initial Development of "Take Home Goal"**: Science panel interaction only. Development of a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force - moderated by Tim Gottwald, Laurene Levy, and Wayne Dixon
10:00 – 10:15 AM	**Break refreshments provided by the Florida Citrus Industry**
10:15 –12:00 PM	**Continuation of Developing Research Priorities List**
12:00 – 1:15 PM	**Lunch provided by the Florida Citrus Industry**
1:15 – 2:45 PM	**Audience Question/Answer Session** – Moderated by Steve Poe and Andy LaVigne
2:45 – 3:00 PM	**Break refreshments provided by the Florida Citrus Industry**
3:00 – 4:00 PM	**Finalizing Research Priorities List** Science panel interaction only
4:00 PM	**Adjourn – Meeting of Steering Committee**

The workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and key representatives for the commodity groups in the citrus industry. Invited scientists are charged with developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The FCCTATF is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida

Steering Committee Co-chairman: Tim Gottwald, Laurene Levy, Wayne Dixon
Members: Richard Gaskalla, Andy LaVigne, Tom Jerkins, Dean Gabriel, Randy Niedz, Michael Hunt, Tim Schubert, Mike Carlton, James Graham, Arnold Tschanz

Citrus Canker Research Workshop
Ft. Pierce, FL
June 20-22, 2000

Mission Statement: development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

Registration: Checklist for invited speakers, moderators, and others. Public Seating will require name and address of attendees.

Breaks and Lunches: Citrus Industry will provide mid-day breaks and lunch to attendees.

Audience Question/Answer Session: 3X5 cards will be available for meeting attendees from 8:00 AM 6/20 to 12:00 PM 6/22. Questions pertinent to research issues are allowed. Questions regarding current program policies and procedures will not be allowed. Members of the Steering Committee will combine duplicate questions. The final stack of cards will be randomly shuffled at the beginning of the Q/A Session. Questions will be read aloud by the moderators and answered by the appropriate scientist(s). At the end of the allotted 2.5 hrs session no further questions will be read from any remaining cards.

Security: The meeting will be held on federal property.

Format of the Workshop: Workshop participants must understand that there will be an environment conducive to frank and candid scientific discussions. Impediments must be identified and effectively solved.

Transcription of Workshop: Professional transcriber will record the workshop proceedings.

Proceedings of Workshop: An internet-compatible (Acrobat Reader file in pdf) proceedings will be published and made available.

Lodging: Radisson Beach Resort, North Hutchinson Island (Ft. Pierce).

Notes to Media Representatives: An information table will be available in the lobby of the meeting facilities. Cameras, video-recorders, tape recorders and similar electronic equipment will not be allowed in the workshop's meeting room. Notes may be taken on paper. A court reporter will record the workshop's proceedings. A copy of the transcript will be available on the Florida Department of Agriculture and Consumer Services.

The workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and key representatives for the commodity groups in the citrus industry. Invited scientists are charged with developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The FCCTATF is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida

Steering Committee Co-chairman: Tim Gottwald, Laurene Levy, Wayne Dixon
Members: Richard Gaskalla, Andy LaVigne, Tom Jerkins, Dean Gabriel, Randy Niedz, Michael Hunt, Tim Schubert, Mike Carlton, James Graham, Arnold Tschanz

Proceedings of the International Citrus Canker Research Workshop

June 20-22, 2000
Ft. Pierce, Florida

Mission

Development of a prioritized list of citrus canker research recommendations for the Citrus Canker Technical Advisory Task Force

Edited and Compiled by:

Wayne N. Dixon, Florida Department of Agriculture & Consumer Services, Gainesville, FL
Laurene Levy, USDA, Animal and Plant Health Inspection Service, Beltsville, MD
Timothy R. Gottwald, USDA, Agricultural Research Service, Ft. Pierce, FL

USDA, Agricultural Research Service
U.S. Horticultural Research Laboratory
2001 South Rock Road
Ft. Pierce, Florida 34945

This document is a pre-meeting compilation of prepared abstracts. A final proceedings will be published after the meeting and made available via the Internet. It will include a transcription of the presentations and discussions as well as the final prioritized list of citrus canker research recommendations.

The International Citrus Canker Research Workshop is an invitational meeting of leading scientists conducting research on citrus canker, scientists and administrators of state and federal regulatory agencies, and representatives for the commodity groups in the citrus industries of Arizona, California, Florida, and Texas. The invited scientists are responsible for developing a prioritized list of research recommendations for the Citrus Canker Technical Advisory Task Force. The task force, appointed by the Florida's Agriculture Commissioner Bob Crawford, is a research, regulatory, and industry partnership charged with the ongoing review of the Citrus Canker Eradication Program to recommend the most appropriate course of action to stop the spread, and ultimately eradicate, citrus canker from the State of Florida.

Steering Committee Co-chairmen
Tim R. Gottwald, USDA, Agricultural Research Service
Laurene Levy, USDA, Animal and Plant Health Inspection Service
Wayne N. Dixon, Florida Department of Agriculture and Consumer Services
Steering Committee Members
Richard Gaskalla, Florida Department of Agriculture and Consumer Services
Andy LaVigne, Florida Citrus Mutual
Tom Jerkins, Blue Goose Growers
Mike Carlton, Florida Citrus Mutual
James Graham, University of Florida
Dean Gabriel, University of Florida
Arnold Tschanz, USDA, Animal and Plant Health Inspection Service
Tim Schubert, Florida Department of Agriculture and Consumer Services
Michael Hunt, Brooks Tropical
Randy Niedz, USDA, Agricultural Research Service

Acknowledgement and appreciation is extended to: (1) the many speakers and moderators who have prepared the following abstracts to promote a scientific dialogue at the citrus canker research workshop and (2) the Florida citrus industry for subsidizing the workshop's refreshments and lunches to maximize the time available for participants to engage in their scientific discussions about citrus canker research directions and needs.

International Citrus Canker Research Workshop
June 20-22, 2000, Ft. Pierce, Florida

An Overview of Argentina's Citrus Canker Control Program with Applicable Costs for a Similar Program in Florida

[1]Ronald P. Muraro, [2]Fritz Roka and [3]Thomas H. Spreen

[1]University of Florida, Citrus Research and Education Center (IFAS), Lake Alfred, FL 333850, USA
[2]University of Florida, Southwest Florida Research and Education Center, Immokalee, FL 34141, USA
[3]University of Florida, Food and Resource Economics Department, Gainesville, FL 32611, USA

Abstract

The best cost effective control of a disease is the absence of the disease. With respect to citrus canker, Florida's current regulatory program is to eradicate the disease. However, the question has been asked: "what would be the cost to Florida's citrus industry if citrus canker became endemic in Florida?" An attempt will be made to address this question recognizing these following limitations:

- Except for eradication, Florida has no experience on effective control programs for citrus canker.
- To address/evaluate "living with canker in Florida" requires assuming that the citrus canker control programs utilized by growers in countries where citrus canker is endemic, e.g., Argentina, would be employed in Florida with similar effectiveness.

The canker control program which will be discussed includes the cost of:

- Additional copper sprays and capital investment for additional spray equipment;
- Windbreaks to limit the movement of citrus canker;
- Possible fruit loss due to canker infestation; and
- "Canker free certification" for specific fresh fruit markets; e.g., Europe.

International Citrus Canker Research Workshop
June 20-22, 2000, Ft. Pierce, Florida

Estimating Spread Of Citrus Canker In Urban Miami Via Differential GPS

[1]Tim R. Gottwald, [2]Xiaoan Sun, [1]Tim Riley, [3]James Graham and [4]Gareth Hughes

[1]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, USA
[2]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL, USA
[2]University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA
[4]Institute of Ecology and Resource Management, University of Edinburgh, SCOTLAND

Abstract

Asiatic citrus canker caused by *Xanthomonas campestris* pv. *citri* (Xcc), continues to spread in South Florida despite efforts by the joint state/federal Citrus Canker Eradication Program (CCEP) to remove and destroy diseased trees. At the request of the CCEP, four areas with low incidence of Xcc in urban Miami were identified to study disease spread. The goal was to measure disease gradients in Miami resulting from rainstorms to determine if the practice of removing exposed trees within 125 feet of diseased trees was adequate to curtail further disease development. To accomplish this, >19,000 trees in dooryards were located via differential GPS and assayed for disease severity, age of infection, citrus cultivar, and canopy size. For each tree, the date the tree became infected was estimated and used to separate trees into contiguous 30-day categories. For each site, distance measurements between focal trees and newly infected trees were calculated for various temporal windows of 30, 60, 90, and 120 days in duration, corresponding to intervals of inspector survey. A Visual Basic routine was used to calculate the distances between each newly diseased tree and all prior focal trees. The nearest distance was used because it was considered to be the most conservative estimate possible. It is therefore likely to be an underestimate of spread, but is a good estimate of the minimum possible distances of spread. Distances of spread were parsed into consecutive 15-meter (50-ft) distance categories and plotted as frequency distributions via a second Visual Basic routine.

In December 1998, results of the epidemiological study were reviewed by a group of scientists and regulatory officials. The consensus was 1) the 125 ft (38 m) radius used to define exposure was inadequate to suppress the spread of canker and ii.) although disease spread was detected up to 58,850 ft (17942 m), the majority of new canker infections occurred within about 1900 ft (579 m) of known source trees. As a result, a new regulation, the ‘1900 ft rule’, was put into practice in late 1999, requiring the removal and destruction of diseased citrus trees and of all citrus trees within a 1900-ft radius of a diseased tree.

Sentinel Tree Survey: A further outcome of the epidemiological study is the adoption of ‘sentinel tree’ arrays of 144 existing dooryard trees of susceptible cultivars in a 12 by 12 arrangement, covering each square mile to be used as an early-warning system for new canker outbreaks. A 15-mile wide by 20-mile long area north of the Dade/Broward outbreak was visually surveyed via this method on repeated 30-day rotations, to detect northward spread toward the major grapefruit production area of the State. To date the method has detected 5 additional canker outbreaks.

Temporal disease progression: Disease progress curves for all four study sites were plotted versus time. Cross correlation analyses were conducted to determine the temporal offset of disease progress for each study site in relation to the weather parameters and indicated that disease was visually detected with the highest accuracy by survey teams ca. 107 days after infection caused by wind-blown rain takes place. A predictive model for disease increase was developed based on the estimated number of existing canker-infested trees, rainfall, and maximum speed of wind gusts with

an $r^2 > 0.96$. The model is being utilized by the CCEP to estimate the number of newly infected trees in the Miami urban area following storm events.

Epidemiology of Citrus Canker in Brazil with and without Asian Citrus Leaf Miner

[1]Armando Bergamin-Filho, [1]Lilian Amorim, [1]Francisco Laranjeira and [2]Tim R. Gottwald

[1]ESALQ – Fitopatologia,13418-900, Piracicaba-SP, BRAZIL
[2]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, USA

Abstract

The citrus leaf miner (*Phyllocnistis citrella*) was first reported in Brazil in 1996. Since then, the citrus canker epidemic was greatly exacerbated in the country, especially in the State of São Paulo where the number of disease foci increased from 25 in 1995 to 45, 190, 457, and 4,180 in 1996, 1997, 1998, and 1999, respectively. As a consequence, 1,737,545 trees were eradicated.

The temporal increase of diseased trees occurred concomitantly with a dramatic change in the spatial pattern of the epidemic, i.e., strongly aggregated patterns, typical of citrus canker from 1957 to 1995, gave way to less aggregated and even at random patterns and presence of satellite foci far away from main foci became very common. This change in the spatial pattern of diseased trees is thought to be caused by the leaf miner, despite the fact that the miner is not a vector of the pathogen. Leaf miner wounds are very susceptible to infection by bacterial aerosols formed during turbulent weather. Leaf miner wounds are very different from natural wounds and are characterized by: (1) a delay in the plant healing reaction (one day for a wound caused by wind, thorns, or pruning versus 1996, 1997, 1998, and 1999, respectively. In 1999, the disease was detected in 299,856 trees. As 10-14 days for wounds caused by the miner); and (2) lower inoculum doses to cause disease (1/100 to 1/1000 of the dose required for infection through natural openings).

Based on the analysis of 203 maps of infected groves just prior eradication, it is proposed that the presence of the miner changes the dispersal function of the disease from a negative exponential model [$y(x)$=a exp(-bx)] to an inverse power law model [$y(x)=ax^{-b}$], where y is disease incidence, x, distance from the source, and a and b are constants determined by regression. This change, according to recent literature based on computer simulation, can explain the patterns seen today in the field.

As a consequence, the eradication rules were changed by a new State law in São Paulo enacted in July 1999. Thus, groves with incidences higher than 0.5% are completely eradicated. Those with incidences below 0.5% have all diseased and exposed trees (in a radius of 30 m) destroyed. In addition, all infected groves are inspected by three independent teams of inspectors. Additional inspections are carried out every 30 days.

International Citrus Canker Research Workshop
June 20-22, 2000, Ft. Pierce, Florida

Survival of *Xanthomonas campestris* pv. *citri (Xcc)* on Various Surfaces and Chemical Control of Asiatic Citrus Canker (ACC)

[1]James H. Graham, [2]Tim R. Gottwald, [2]Tim D. Riley, [1]Jaime Cubero and [1]D. L. Drouillard

[1]Universty of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA
[2]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, USA

Abstract

Survival on Various Surfaces: Compliance agreements have now been issued statewide in Florida for decontamination of personnel and equipment which focuses attention on the situations of greatest risk for Xcc survival and transmission. *Xcc* survival is evaluated on various materials, including wood (representing crates, ladders), cotton cloth (clothing), cotton gloves, plastic (fruit crates), metal (vehicles, lawnmower blades), leather (gloves, shoes), feathers (bird), and fur (animal). Bacterial inoculum is prepared from macerated ACC lesions and applied to the various surfaces to be tested. These surfaces are exposed to ambient meteorological conditions, in sun or shade under at the containment greenhouse/laboratory facility at Opa-locka Airport in Miami. Survival is significant up to 48 under sun and 72 hours under shade depending on the weather conditions during the test (e.g., temperature, humidity). This confirms that when surfaces are dry, *Xcc* dies, but before drying there is a considerable period of risk of bacterial transmission. In Miami, where diseased trees are chipped after removal from dooryards, the debris produced is laden with *Xcc* detectable by air sampling in the vicinity (10-20 ft.) of the machinery. Aerosol inoculum is also capable of causing infection of wetted foliage located in the zone of bacterial dispersal. This finding led to procedural changes for when and where chipping can be safely conducted to minimize risk of bacterial dissemination in the area of tree destruction.

The investigators have developed the capability to detect and quantify *Xcc* by a competitive PCR utilizing DNA primers developed by Dr. John Hartung, USDA/ARS, Beltsville, MD. Comparison of PCR methods with plating on selective medium to detect culturable bacteria will enable us to ascertain whether non-culturable and or nonviable cells occur on or in plant material since the PCR primers will detect DNA from intact bacteria living or dead. If non-culturable bacteria are detected, the epidemiological potential of this population will be will be evaluated under a wide range of meteorological conditions. The significance of detection of non-culturable or dead *Xcc*, with DNA probes in quarantine situations will be put into proper perspective.

Chemical Control: Four induced systemic resistance (ISR) compounds are under evaluation for their potential to control ACC using citrus bacterial spot (CBS) on Swingle citrumelo, caused by *Xanthomonas campestris* pv. *citrumelo,* as a surrogate pathosystem. ISRs currently under evaluation are Messenger (a.i. Harpin, Eden Bioscience), Nutri-Phite (phosphite, Biagro Eastern), Oxycom (activated oxygen radical, Redox Chemicals Inc.), and FNX-100 (phosphite/Phosphate, Foliar Nutrients Inc.). Messenger significantly reduces the number of lesions per injection site after inoculation of expanding Swingle citrumelo leaves. Messenger, Nutri-phite, and FNX-100 are also under evaluation in two Florida field nurseries with recurring CBS disease epidemics. Compounds with activity will be tested on ACC-infested trees under containment in Miami and in field plots outdoors at this facility if a current proposal to do so is approved by the Citrus Canker Eradication Program (CCEP). Promising compounds are proposed for testing in cooperation with Dr. Rui Leite, IAPAR, in Parana State, Brazil during the coming 2000-2001 season.

Control Studies In Brazil

Rui P. Leite, Jr.

Instituto Agronômico do Paraná - IAPAR, 86001-970 Londrina-Parana, Brazil

Abstract

Citrus canker caused by *Xanthomonas axonopodis* pv. citri was first reported in Brazil in the western region of São Paulo state, in 1957. Since this detection, quarantine restrictions and eradication measures were enforced to eliminate the disease and to prevent the spread of the pathogen to new areas. However, these efforts were not enough to eradicate the disease nor to contain its spread. Citrus canker has already been detected in the major citrus-growing areas of several states of the Central and Southern regions of Brazil, including Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, Mato Grosso, Goiás, São Paulo, and Minas Gerais.

Research studies on the control of citrus canker have been carried out by Instituto Agronômico do Paraná and Instituto Biológico de São Paulo, as early as the late 1970s. These research programs have addressed several aspects for more effective management of citrus canker, such as evaluation of citrus cultivars for resistance, chemical control, and cultural practices. A wide range of reaction to citrus canker was observed among citrus cultivars of sweet oranges, mandarins, and limes under field conditions. Citrus germplasm has been broadly classified into six classes with regard to the reaction to citrus canker. Several commercially important cultivars, such as Orlando tangelo, Marsh seedless grapefruit, Mexican lime, Siciliano lemon, and Hamlin, Bahia and Baianinha sweet oranges, are among the most susceptible. On the other hand, some widely planted cultivars, e.g., Ponkan, Satsuma and do Rio mandarins, Tahiti lime, and Pera, Folha Murcha and Valencia sweet oranges, are among the most resistant.

Several experiments have been carried out to evaluate timing and formulation of bactericides to control citrus canker under field conditions. Copper compounds are the only chemicals which have shown some degree of control of the disease. Sprays of copper during the growth season to protect new flush and young fruits of infection by the bacterium have reduced significantly disease incidence on leaves and fruits. Windbreaks of silk oak have also been examined for the control of citrus canker. The combination of practices, such as use of resistant cultivars, copper sprays, and windbreak, has also been extensively evaluated for citrus canker prevention and control. Based on the results of the aforementioned research, an integrated citrus canker management program has been devised to prevent the introduction and to control the disease in citrus orchards in southern Brazil.

Citrus Bacterial Canker Disease: A Review on Research Developed on Réunion Island (France)

Olivier Pruvost, Christian Verniere, Annie Couteau, Bernard Boher, and Michel Nicole

CIRAD, Phytopathology Laboratory, Saint Pierre 97410, REUNION, FRANCE

Abstract

Citrus bacterial canker (CBC) is an economically important disease in many tropical and subtropical countries. In most of these countries, the disease has been occurring for decades and no eradication programs are currently being conducted. The main objective of our group is to improve the knowledge on CBC and its causal agent relative to tropical areas. The main results obtained during the last decade deal with: (1) the characterization of the causal agent, *Xanthomonas axonopodis* pv. *Citri* (Xac), in the Indian Ocean Region, and (2) an analysis of CBC development in Réunion Island (survival of Xac; dynamics of Xac exudation from lesions; and spatio-temporal analyis of CBC development in experimental plots).

All strains isolated so far in the Indian Ocean Region share more similarities with *Xanthomonas axonopodis* pv. *citri*, the causal agent of Asiatic citrus canker (A canker), than with *Xanthomonas axonopodis* pv. *aurantifolii*, the causal agent of South American canker (B/D/D canker). However, most strains isolated in the Mascarene archipelago have atypical β-lactam antibiotic susceptibility profiles. Some strains isolated from India have a limited host range and constitute a new serogroup.

The negative influence of mild winter temperatures, generally prevailing in the lowland tropics, on Xac populations in leaf lesions was evaluated. As expected, the decrease in populations was much less pronounced than those previously recorded in Japan and Argentina. A study of the dynamics of internal and excluded Xac populations from lesions differing in age and submitted to simulated rainfall globally indicated that the biological significance of old lesions as inoculum sources is greater in the lowland tropics. A histological study of canker lesions indicated an accumulation of phenolics in 6-10 month old lesions. However, large amounts of bacterial cells embedded into a polysaccharidic pink-stained material were observed in intercellular spaces of the paravascular parenchyma and spongy parenchyma at the margin of old lesions, indicating that old lesions are still active.

An overview of problems encountered in tropical countries for controlling ACC will be presented.

Efficacy of Bactericides for Control of Citrus Canker and for Reducing Epiphytic Populations of *Xanthomonas campestris* pv. *citri*

L. W. Timmer

University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA

Abstract

Bactericides were evaluated in trials in nursery plantings of sweet orange and rough lemon and on mature grapefruit trees. Copper-containing materials were generally superior to antibiotics and other products in reducing epiphytic populations of *Xanthomonas campestris* pv. *citri* (Xcc) and the number of lesions on leaves and fruit. Copper ammonium carbonate was generally more effective than other copper products. Application of copper ammonium carbonate to existing foliage reduced epiphytic populations of Xcc and the number of lesions on subsequent flushes of growth. In field trials in Argentina on navel oranges and grapefruit, from 3 to 5 applications of copper-containing products from August to January provided moderately good control of citrus canker.

Inoculum Production and Epiphytic Survival of *Xanthomonas campestris* pv. *citri*

L. W. Timmer

University of Florida, Citrus Research and Education Center Lake Alfred, FL 33850, USA

Abstract

Inoculum production and release of *Xanthomonas campestris* pv. *citri* (Xcc) lesions of Asiatic citrus canker was studied by attaching wells on grapefruit leaves surrounding young lesions and adding water. About 10^4-10^5 bacteria were exuded immediately and cumulative release over 24 h ranged from 10^5 to 10^6 per lesion. Fewer bacteria were exuded and bacteria were exuded more slowly from old lesions. Populations of Xcc on canker-affected leaves and asymptomatic leaves were assayed using a leaf swab technique in a nursery of symptomatic Duncan grapefruit seedlings and on potted asymptomatic trap seedlings. Following rainfall or irrigation, Xcc populations ranged from 10^5 to 10^7 colony-forming units (cfu) per leaf on symptomatic and from non-detectable to 10^5 cfu per leaf on asymptomatic leaves. Recovery of bacteria was highest in the early morning and declined as the moisture from dew dried. Populations in droplets of bacterial suspensions dried onto the leaf surface declined with no evidence of multiplication even when moisture was restored. The potential infectivity of epiphytic population was tested, but residual epiphytes did not appear to contribute to infection. Citrus canker lesions exude bacteria in the presence of moisture which are splash-dispersed to asymptomatic leaves where they survive as epiphytes but do not appear to multiply.

Citrus Canker In Argentina - Control, Eradication, And Current Management

Blanca Isabel (Nelly) Canteros

Estación Experimental Agropecuaria Bella Vista, Instituto Nacional de Tecnología Agropecuaria INTA, SAGPyA, ARGENTINA

Abstract

In 1999, fifty million citrus plants were grown in Argentina. Approximately 65% of the citrus is in the northeastern Argentina with 50% as orange cultivars, 41% tangerine, 6% lemon, and 3% grapefruit. Citrus canker B was introduced into Argentina in 1928; however, its origin was not traced. Low agressivity and restricted host range confined it for 40 years in a small area. Citrus canker B disappeared in 1978-90 after the introduction of the more aggressive Asian strain of citrus canker, which was present in Brazil in 1957 and Paraguay in 1965. In bordering areas of Argentina, Asian strain citrus canker infections started in 1974. Spread of this strain was relatively rapid from 1977 to 1980 followed by a slower but relatively constant expansion through Argentina. By 1990, Asian strain citrus canker was considered endemic. It has moved to all available citrus plants in northeastern Argentina, even isolated urban trees. Northwestern Argentina, separated from the Northeast by dry areas of woodlands, is free of canker.

A citrus canker program was initiated in 1977 with 600,000 mature citrus trees destroyed. In Corrientes, only highly infected plants (50% intensity) were cut (200,000), most of these were grapefruit. In Entre Ríos, all affected plants (400,000), except lemons, were destroyed. From 1977-78, 2.5 million trees were surveyed in Corrientes: disease incidence (% infected plants) was 22% and intensity was 11%. Citrus in Corrientes in 1977 numbered 6.5 million plants with 60% as orange cultivars, 20% grapefruit, 10% lemon, and 10% tangerine; in 1999 there were 10.5 million trees with 60% orange, 30% tangerine, 8% lemon, and 2% grapefruit.

With the INTA-IFAS Cooperative Canker Project (1978-1983), timing of sprays was set and methods of research were developed. Studies were continued at INTA Bella Vista Station. Canker intensity variation was described as the effect of environmental factors, important even in plants without chemical sprays. The intensity in each tree and severity on fruits vary with the seasons and are lowest on those years with low rainfall in the spring. Variations are similar regardless of host resistance. Location of EEA INTA Bella Vista and Weather Station, NE Argentina, at 28° 26'S; 58° 55'W; 70 m above sea level, annual rainfall 1179 mm, sd 275, and 83 rainy days, sd 12.5. In low infection years, only grapefruit differs from other cultivars in canker intensity on fruits. Important factors are temperatures, relative humidity, and, most important, rain and wind taken together. The ENSO phenomenon is responsible for the cyclic variation of the disease.

Total loss in citrus productivity is low. The cost of sprays is not high compared to other pests. Several years after the spread of citrus canker, planting was resumed with the advantage of the use of modern cultivars. The most important economic loss is caused by the quarantine restrictions on fruit from citrus canker-infested area as imposed by citrus canker-free citrus-growing countries. Conclusive results on fresh fruit as a mechanism of disease spread are not available.

Since 1994, as part of the National Program of Citrus Health (implemented by the National Plant Health Agency, provincial government, citrus growers and contributors of INTA) an Integrated Plan to Reduce the Risk of Canker has been underway in Northeast Argentina. After 30 years of research, it was apparent that it is possible to obtain canker-free fruit with the use of the correct management. Requirements to export fresh fruits includes certification of symptom-free plots. Populations of *Xanthomonas axonopodis* pv. *citri* (Xac) were found to be low even from highly infected plots of different varieties in lesionless leaves and fruits, and almost always undetectable in low disease intensity groves. Detection was by washing and semi-selective medium or infiltration of leaves. A positive linear relationship exists between inoculum concentration and number of lesions per square centimeter in fruits. Resistance is expressed, as in leaves, as lower number of lesions per unit area. Susceptibility is highest in fruits such as 'Murcott' until 25% final size; 'Valencia' to a 40% size, grapefruit to a 55 % size , and 'Navel' to a 63% final size.

Efficacy of disinfectants to exclude any epiphytic Xac was also determined. Detection was by washing, plating, and infiltration of leaves. Effects *in vitro* and on artificially infested fruits were investigated. Commercial SOPP (2% for 2 minutes) and SH (0.02% for 45 seconds) were the best treatments on fruit from infected plots. Both treatments together killed high concentrations of Xac when applied on fruit surfaces. The same products were highly effective against Xac *in vitro* even at very low rates (SOPP 0.03% and SH 0.001%). Regulations to produce fruit in symptom-free plots are in efffect. The participation of growers is voluntary. Certified packing houses should treat fruit with approved disinfectants.

Windbreaks are required around a 2-4 ha plot and every 100 meters in windy locations.

Control is required of the Asian citrus leaf miner (*Phyllocnistis citrella* Sta.) that entered Argentina in 1996 and became widespread in very short time. Leaf miner damage is important to canker infection as in Southeast Asia. However, the occurrence of canker lesions on leaves with leaf miner damage was evident only on infected trees whereas disease-free plants did not show new lesions, even those heavily damaged by the insect.

Biological and chemical control are in effect. In plants with severe canker, heavy defoliation occurs after leaf miner damage and heavy sprouting will follow. Finding the best treatment combination for both is discussed. Pruning of affected tissue decreases the available inoculum sharply and is used in Argentina in symptom-free plots. Herbicide defoliation can be used to start a program. Equipment, hands, clothing and gloves of laborers, collecting boxes and any other tools should be treated with disinfectants such as quaternary ammonium, fosforic acid-iodine solutions, Na hypochlorite or 70% ethanol. All young citrus tissue should be treated to prevent infection. Sprays are applied to leaf flushes in their susceptible stage (10 to 14 days old) and to developing fruits every 40 days. Recommended chemicals are copper products. Soluble powders are preferred over liquid forms. The most important sprays are those applied from bloom to four months later. Timely application of copper sprays provided excellent control of the disease until 1994 when lack of control was noted in several groves and nurseries. Copper resistance was demonstrated and the mixture of copper+mancozeb was the best in vitro and in field trials. Current recommendations include the addition of mancozeb to the copper aprays in groves where resistance is found. Results obtained are excellent.

Methods for Detection and Characterization of *Xanthomonas axonopodis* pv. *citri*

[1]Jaime Cubero, [1]James H. Graham and [2]Tim R. Gottwald

[1]University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA
[2]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, USA

Abstract

The importance of accurate and rapid diagnosis of citrus bacterial canker has prompted the development of efficient detection and characterization methods for *Xanthomonas campestris* pv. *citri* (Xcc) to be applied in the eradication program and for epidemiological purposes.

Presence of compounds in plant material that inhibit PCR reactions is one of the major limitations for the application of this technique in a routine diagnosis. To address this drawback, an internal control (IC) for the PCR reaction was designed. IC is based on a plasmid that contains a fragment with the sequences needed for amplification with a set of primers previously described by Dr. Hartung to identify Xcc. The plasmid added to each PCR reaction performed generates, after amplification, a product of 400 bp clearly different from the 222 bp product obtained from the target sequence on a plasmid in Xcc. The inclusion of this internal control ensures the attainment of at least one amplified product but only if the DNA extraction is of high enough quality to be amplified. The inclusion of IC in the reactions eliminates the risk of false negative results.

To characterize and discriminate among Xcc strains, two different approaches were followed. In the first approach, the ribosomal DNA operon was analysed. A region that included the small subunit 16S, the intergenic transcribed spacer (ITS) and a portion of the 23S was amplified by PCR and digested with several restriction enzymes and finally this region was cloned and sequenced. However, no difference in the ITS sequence observed between the two strains of Xcc originally isolated from Dade Co. and Manatee Co. in Florida. In the second approach, repetitive DNA PCR (rep-PCR) was applied. Intervening DNA between two adjacent repetitive elements in the genome of Xcc were amplified by PCR using primers that anneal specifically to these repetitive sequences. Differences in fingerprints of rep-PCR products from different strains are being used to discriminate strains from Florida and also to characterize other *Xanthomonas* strains from citrus in a worldwide collection in Beltsville, MD.

Integrating the Scientific Perspective into Citrus Canker Eradication Efforts in Florida

Tim S. Schubert, Xiaoan Sun, and Wayne N. Dixon

Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32614, USA

Abstract

The Florida citrus canker eradication program relies heavily upon accurate, up-to-date, and locally applicable scientific information to guide the process. This information provides the basis for program decision making, but it must be integrated with legal, logistical, financial, public relations, cultural, and political perspectives in the process of carrying out the actual detection and eradication. This integration process has resulted in some noteworthy developments that serve to broaden the perspectives of plant pathologists involved in the program. One result of the integration of perspectives outside of the scientific arena is that the scientists are often asked to provide second and third choice options that may lessen the overall chances for swift eradication and prolong the duration of the program. However, such options are considered essential to make the eradication effort feasible at all. Some of the subject areas that will be discussed for both residential and commercial settings are: 1) survey and detection methods; 2) field and laboratory diagnostic procedures; 3) disinfection/sanitation practices; 4) tree removal techniques; 5) transportation and disposal methods for diseased and exposed citrus waste; 6) determination of exposure zones; and 7) risk assessment procedures.

Citrus Canker Detection and Diagnosis in Florida

Xiaoan Sun, Tim S. Schubert, Wayne N. Dixon, and Maria Peacock

Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32614, USA

Abstract

Asiatic citrus canker, caused by *Xanthomonas axonopodis* pv *citri* (Xac), was initially found in urban Miami in 1995 and has spread to the residential areas and commercial groves of seven counties in Florida. Detection of new disease infections still depends on visual examination of citrus. Current citrus canker diagnostic protocol requires that a sample from the first canker find in a square mile area (section) be submitted to the quarantine facilities in Gainesville for a series of laboratory tests. After the lab confirmation, the subsequent finds in the same area are determined by a field plant pathologist/canker diagnostician. To date, all citrus canker samples, which were confirmed in the field and sent to the quarantine laboratory, have been tested positive for citrus canker in the quarantine laboratory. Microscopic examination of canker lesions for typical symptoms and for bacterial streaming, coupled with a positive pathogenicity test on Duncan grapefruit and positive hypersensitivity test on tomato using a pure culture of the isolated pathogen, are routinely used for every lab diagnosis of citrus canker. The recent find of a unique isolate of an apparent citrus canker in Wellington and Lake Worth, Palm Beach Co. has suggested that additional laboratory tests, such as ELISA, PCR, plasmid analysis and fatty acid analysis, be utilized if any deviation or irregularity from regular host range or a typical A-strain canker symptom are observed in the field or laboratory.

Detection of an Unique Isolate of Citrus Canker Bacterium from Key Lime in Wellington and Lake Worth, Florida

[1]Xiaoan Sun, [1]Robert E. Stall, [2]Jaime Cubero, [3]Tim R. Gottwald, [2]James. H. Graham, [1]Wayne N. Dixon, [1]Tim S. Schubert, [1]Maria E. Peacock, [4]Ellen R. Dickstein and [5]Paul H. Chaloux

[1]Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Gainesville, FL 32614, USA
[2]University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850, USA
[3]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, USA
University of Florida, Department of Plant Pathology, Gainesville, FL32611, USA
USDA Citrus Canker Eradication Program, Plantation, FL 33313, USA

Abstract

In Wellington and Lake Worth areas, citrus canker has reportedly caused the disease in key lime over a period of three years. But other adjacent citrus varieties, such as grapefruit and sweet orange were unaffected. The causal bacterium was easily isolated form leaf and stem lesions. It grew on nutrient and lima bean agar. Colonies appeared similar to those of Asiatic citrus canker (A-strain citrus canker) bacterium grown on nutrient agar, but the bacterium appeared less gummy on lima bean agar slants. When citrus plants were inoculated with pure cultures of the bacterium, typical A-strain citrus canker symptoms developed on key lime. However, on Duncan grapefruit, a brownish, flat, and necrotic lesion was produced. We are conducting a series of tests, including ELISA, PCR, plasmid analysis and fatty acid analysis, to determine the relationship of this strain to the A-strain citrus canker, caused by *Xanthomonas axonopodis* pv *citri* (Xac).

Analysis of Fatty Acid Profiles of Xanthomonads Pathogenic to Citrus

[1]Robert E. Stall, [2]Ellen R. Dickstein and [2]Jeffrey B. Jones

[1]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32614, USA

[2]University of Florida, Department of Plant Pathology, Gainesville, FL 32611, USA

Abstract

Fatty acid analysis has been used in bacterial identification for over 35 years. Methylation of the fatty acids (FA) extracted from bacterial cells facilitates the identification and quantification of the FA by gas chromatography. The fatty acid profiles from members of the bacterial genus *Xanthomonas* are relatively complex compared to those of other plant pathogenic bacteria. More than 20 FA occur in *Xanthomonas* and the differences in relative quantity of them among strains presents the potential for distinguishing groups of strains within the genus. Strains can be grouped according to FA content with the statistical programs associated with software developed for the Microbial Identification System. Using principal component analysis and generating a 2-D plot, the Asiatic strains of citrus canker form a discrete cluster and can be distinguished from strains of the citrus bacterial spot pathogen, which form a separate cluster. In preliminary tests and using the same techniques, the strains of Asiatic canker from Miami were distinguished from the Manatee strains. Furthermore, the strains of citrus canker from Key Lime in Palm Beach County were also differentiated from the Asiatic strains of citrus canker in Florida. Determination of fatty acid profiles through gas chromatography may be a rapid and sensitive method for identification and separation of strains of *Xanthomonas* on citrus in Florida.

Xanthomonas axonopodis pv. *citri* Genome Project

Jesus A. Ferro

Departamento de Tecnologia, Laboratório de Bioquímica e de Biologia Molecular, Universidade Estadual Paulista-UNESP, 14.870-000 - Jaboticabal, SP, BRAZIL

Abstract

A network of 13 laboratories in the State of São Paulo, Brazil, is sequencing the genome of the plant pathogen *Xanthomonas axonopodis* pv *citri*, the causative agent of Asiatic citrus canker in Brazil and many others countries in the Americas, Southern Asia, Japan, the Middle East and Africa. The project is funded by São Paulo State Research Foundation (FAPESP) and by the Citrus Plant Protection Fund (FUNDECITRUS), an association of the citrus industry and producers.

The sequence strategy involves: 1. End sequencing of shotgun clones (1-4 Kbp) generated from several libraries produced in the two central laboratories; 2. End sequencing of two cosmid libraries and 3. PFGE restriction mapping of genomic DNA. The sequencing effort began in September 1999 and the genome is already virtually closed, with a 25-fold genome coverage by shotgun reads. The preliminary genome assembly indicates a genome size between 5.1-5.2 Mbp and the assembly is consistent with the restriction map obtained by PFGE. The virtual gaps will be closed by sequencing cosmids or shotgun clones.

Although annotation (gene identification and description) will begin in August of this year, some features of the genome have been already disclosed: 1. The restriction map of X. citri strain is different from those described for *X. campestris* pv. *campestris* (J. Bacteriol. 181:117-125, 1999) and *X. axonopodis* pv *glycines* (FEMS Microbiol. Lett.175:59-68, 1999); 2. The G+C content is 64.7% (*Xylella fastidiosa* is 52.67%); 3. Two circular plasmids were identified, having approximately 60 and 30 Kbp; 4. Avr genes (ptha) were mapped by hybridization in the largest plasmid. In addition to the complete sequencing the *X. axonopodis* pv *citri* genome, a partial sequencing of the *X. campestris* pv *campestris* genome will be carried out for comparison. Completion of the project is planned for March 2001 and the entire annotated database will be publicly available upon publication of the results.

Detection of *Xanthomonas axonopodis* pv. *citri* Bacteria and Differentiation of Strains

John S. Hartung

USDA, Agricultural Research Service, Fruit Lab-PGQO, Beltsville, MD 20705, USA

Abstract

Several distinct groups of Xanthomonads incite diseases of citrus. These can be differentiated by host range within citrus (broad to narrow), symptomology (hypertrophy and hyperplasia or water-soaking), physiological tests, and sensitivity to bacteriophages. Such phenotypic traits are correlated with a clonal population structure revealed by RFLP analyses of both genomic and plasmid DNA. Detection methods include isolation on microbiological media followed by physiological testing, plant inoculations to induce symptoms, ELISA, hybridization tests and various tests based on the polymerase chain reaction. The most widespread form of citrus canker, the 'Asiatic' canker, caused by *Xanthomonas axonopodis* pv. *citri* can be detected at single cell sensitivity by the polymerase chain reaction. We are currently adapting this PCR assay to the light cycler technology. This will provide very rapid and quantitative detection of Xanthomonads associated with citrus. Illustrations of the current and existing technology will be presented, as well as suggestions on where improvements must be made, and where uncertainties exist in the area of strain differentiation and classification.

Remote Sensing and Spectral Analysis: A Moderated Discussion Session

[1]Chenghai Yang and [2]Marcus Borengasser

[1]USDA, ARS, Integrated Farming and Natural Resources Research Unit, Westlaco, TX 78596, USA
[2]Midwest Research Institute, GIS/Remote Sensing Division, Palm Bay, FL 32909, USA

Abstract

- Principles of remote sensing (concept, electromagnetic spectrum, reflectance curves)
- Remote sensing systems (satellite vs. airborne; photography vs. digital imagery; multispectral vs. hyperspectral)
- Global Positioning Systems (GPS) and image rectification
- Application examples (citrus blackfly, citrus greasy spot)

- Introduction to hyperspectral remote sensing
- Field spectroscopy and reference spectra
- Spectral reflectance examples from Siboney
- Image exploitation in a GIS
- Introduction to high-resolution spaceborne systems

Discussion Topics

Comparison of hyperspectral platforms

Platform	Scale Range	Data Type	Coverage
Vehicle %	tree to entire tree	spectra	trees/hr
Airborne	sub-meter or greater	imagery	20K acres/hr
Spaceborne	5 meters/30 meters	imagery	30km swath

Necessary resources for users

Platform	Minimum	Optimum
Vehicle	grove map	GIS software
Airborne	GPS, image viewer	image processing software
Spaceborne	GPS, image viewer	image processing software

User needs vs. available products and services

Technology transfer

Economics

Relevance to precision agriculture

Integration into GIS

Relevance to other citrus diseases

Other sensors

Detection Technology - A Polymer Composite-Based Electronic Nose: A Moderated Discussion Session

Michael E. Koscho and Nathan Lewis

California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA 91125, USA

Abstract

A simple, broadly responsive detector array, based on polymer-carbon black composites will be described. Due to parallels which can be drawn between such arrays and mammalian olfaction, arrays of sensors of this type are generally referred to as "electronic noses." Humans (and other mammals) are thought to have on the order of 1000 different olfactory genes, each of which encodes a specific receptor protein. There is mounting evidence that these receptors do not employ a lock-and-key type specificity for individual odorants. Rather, individual olfactory receptors are broadly responsive to a number of odorants. Additionally, a particular odorant will afford responses in a number of receptors. It is the pattern of responses which is believed to afford the sense of olfaction, much in the same way an array of broadly responsive sensors is capable of classifying vapors.

Each of the detector elements of our "electronic nose" is composed of a thin film of conducting, carbon black particles dispersed in an organic polymer. Swelling of the polymer upon vapor sorption gives rise to an increase in the electrical resistance of the polymer-conductor composite, which can be monitored using simple, low-power electronics. By varying the organic polymer used to fabricate each element of the array, each has the potential to differentially respond to a specific vapor producing a pattern of responses which is distinct for that particular vapor, and the extent of the sensor responses allows quantification. Basic array construction, response characteristics, data manipulation, and issues of sensitivity and selectivity will be presented.

Citrus Breeding And Transformation: A Moderated Discussion Session

[1]José X. Chaparro, [2] Vicente J. Febres and [2]Gloria A. Moore

[1]USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, Fl. 34945, USA
[2]University of Florida, Department of Horticultural Sciences, Gainesville, FL 32611, USA

Abstract

The majority of commercial citrus genotypes are of hybrid origin and do not represent pure species. The diversity of morphological phenotypes appears to have arisen through somatic mutation as evidenced by the difficulty in developing sweet orange (*Citrus sinensis* (L.) Osbeck) cultivar specific markers. However, development of molecular markers is allowing a preliminary comparison of established classification groupings and those generated from marker data. The data provides evidence of the parental species involved in the origin of hybrid genotypes. All species belonging to the *Citrus* genus that have been tested are susceptible to the Asiatic citrus canker strain, with grapefruit (*C. paradisi* Macf.) and Mauritius papeda(*C. hystrix*) being the most susceptible. Resistance to Asiatic citrus canker may be available in two distantly related species: 'Nagami' kunquat (*Fortunella margarita* (Lour.) Swingle) and Chinese box orange (*Severinia buxifolia* (Lam.) Jack). However, introgression of resistance genes from either of the two species into commercial germplasm using traditional breeding techniques is likely to be difficult and time consuming due to the poor quality of the fruit produced by each species.

Progress has been made in understanding the genetic basis of plant disease resistance by the use of genome mapping techniques. The use of markers and appropriate screening procedures will allow the identification of the genetic locus/i required for resistance and the location of the locus/i in the genome. Similar information has been used to map the location of the Citrus Tristeza Virus (CTV) immunity originating from trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and to demonstrate that it is controlled by a single genomic region. The information has been the source of molecular markers used for selection of CTV resistant genotypes. Genome map information is the primary information used in current research to clone the CTV immunity locus. Preliminary results indicate that the genome region containing the CTV immunity locus has additional sequences with homology to disease resistance genes from other plants. Similar procedures could be used to determine the genetic basis of resistance and clone the resistance gene from 'Nagami' kumquat for use in citrus.

Genetically modified plants are currently used in a number of different ways. The most important commercial use of transgenic plants is to accelerate plant breeding since one or a few useful genes can be transferred directly into an otherwise ideal variety. Although this can also be achieved by conventional plant breeding, it involves several generations of backcrosses, which in Citrus could take decades. Therefore, with genetic transformation, it is possible to target a specific problem affecting plants, if the genetics affecting the particular situation are sufficiently characterized.

A method for the routine genetic transformation of epicotyl segments from etiolated citrus seedlings using *Agrobacterium tumefaciens* has been developed in our laboratory. This model system uses grapefruit (*Citrus paradisi* cv. Duncan) with an efficiency of 44% of the regenerated

shoots being transgenic. Approximately 12% of the regenerated shoots are solid transgenics with the rest being chimeric. A set of plasmids has been developed with different selectable marker genes (GUS and GFP) and promoters (cauliflower mosaic virus 35S and figwort mosaic virus 34S) to facilitate cloning, gene transfer and expression in plants. With these tools it is possible to easily and quickly transfer any sequence into grapefruit. These techniques have been used to transform citrus with several structural and non-structural genes of the citrus tristeza virus in an attempt to develop resistant plants. In addition, cold-regulated sequences and other metabolic genes are being used to improve plant and fruit quality. Altogether, several hundred transgenic plants have been produced. Mexican lime and Carrizo citrange have also been transformed using this protocol.

Finally, efforts are currently under way to develop a procedure for the transformation of mature tissue that will reduce the juvenility stage of the transgenic plants. Thus, the technology is in place to apply genetic transformation techniques to the citrus canker problem. What is needed are genetic strategies to test.

Discussion on Genomics, Resistance and GMOs: A Moderated Discussion Session

[1]Dean Gabriel and [2]John Hartung

[1]University of Florida, Department of Plant Pathology, Gainesville, FL 32611, USA
[2]USDA, Agricultural Research Service, Fruit Lab-PGQO, Beltsville, MD 20705, USA

Abstract

Sustainable long term control of citrus canker and other citrus diseases will depend on exploiting comprehensive genomic information derived from both the pathogen and citrus. Fortunately, full sequence information is or will soon be available for several citrus pathogens including *Xanthomonas axonopodis* pv. *citri*. Two approaches aimed at gaining sequence information on the citrus host will be discussed. One approach would be to immediately make cDNA libraries from citrus and sequence portions of up to 10,000 clones. This information would be exploited by Sequential Analysis of Gene Expression, a technique which provides many of the benefits of gene chip technology at a fraction of the cost. A longer term goal should be to obtain complete sequence information for a citrus variety. An international strategy to reach this goal based on the successful Brazilian model of a 'virtual genomics institute' will be described. Feasibilities, approximate costs, and benefits to the industry will be discussed for both approaches, with emphasis on the impact on citrus canker and other exotic citrus diseases.

Different options for using genetically modified organisms to control citrus canker disease will also be discussed. One approach would be to engineer the host for specific resistance to *Xanthomonas axonopodis citri* using information about the role of the *pth*A gene in pathogenesis. Another approach would be to engineer viruses of citrus to express antimicrobial peptides. Selection criteria for both the viruses and antimicrobial agents will be discussed. Citrus canker has created a crisis for the Florida citrus industry. If biotechnological approaches to disease control could be shown to be safe and effective, they could have a major positive impact in this situation. The positive impact could potentially extend beyond citrus into the larger debate on GMOs and agriculture. Unusual aspects of the citrus canker situation in Florida that potentially favor a biotechnologiucal approach will be discussed.

Citrus Canker Epidemiology – Methodologies and Approaches: A Moderated Discussion Session

[1]Armando Bergamin-Filho and [2]Gareth Hughes

[1]ESALQ – Fitopatologia,13418-900, Piracicaba-SP, BRAZIL
[2]Institute of Ecology and Resource Management, University of Edinburgh, Edinburgh EH9 3JG, SCOTLAND, UK

Abstract

The primary goal of epidemiology is to increase our knowledge of how diseases develop in host populations in both space and time. With improved knowledge about these processes, epidemiologists can propose more efficient and effective strategies and tactics for disease control.

Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri*, is one of the most important diseases of citrus. Despite this, the epidemiology of citrus canker is not well understood in both spatial and temporal aspects. This is probably due to the method of control usually adopted, i.e., when citrus canker is found, every effort is made to prevent further spread of the pathogen and, if possible, to eradicate it by destroying all infected and 'exposed' trees. This approach is used both in Florida (USA) and São Paulo (Brazil), two of the main citrus-producing areas in the world. Thus, epidemiological studies of the citrus canker pathosystem in Florida and São Paulo are restricted to spatial data collected prior to tree destruction.

The basic facts about the citrus canker disease cycle are well known. Leaf infections occur through stomata and wounds. Leaves are most susceptible when expanded between 50 and 80%. Rain wets disease lesions on citrus fruits, twigs, and leaves, allowing the bacteria to ooze to the plant tissue surface. High wind speeds during rain cause water-soaking and facilitate entry of bacteria through stomatal openings into leaves. Wounds caused by the Asian citrus leaf miner (*Phyllocnistis citrella*) can delay substantially the healing reaction of the affected cells. This type of wound remains susceptible for 7 to 14 days compared to only 24 hrs for wounds caused by wind, thorns, or pruning. Infection through wounds needs only 1/100 to 1/1000 of the inoculum dose required for infection through stomatal openings.

Of the various temporal models tested for citrus canker (linear, monomolecular, exponential, logistic, Gompertz), the Gompertz model usually gives the best overall fit. Rates of disease increase depend on many factors (including cultivar, weather, and management practices) and ranged from $k = 0.005$ to 0.24 per day in several citrus canker experiments in Argentina. Drip or mist irrigation limits the development of citrus canker compared with overhead irrigation, which causes splash dispersal of inoculum. Rates of disease increase are statistically higher in plots with overhead irrigation than in plots with either drip or mist irrigation, as shown in Reunion Island.

It is well established in the literature that the dissemination of *X. axonopodis* pv. *citri* is directly related to wind-driven rain, especially when wind speeds exceed 8 m/s, mostly over short distances, i.e., within trees or to neighboring trees (long-distance spread occurs with the movement of diseased propagating material). Consequently, the spatial pattern of the disease is highly aggregated irrespective of prevailing weather, management practices or irrigation system utilized. Aggregation is usually maintained and often accentuated over time. Spatial

autocorrelation analyses usually indicate an absence of, or few, reflected clusters, of small size, suggesting that relationships among individual foci did not exist or were not strong. Slopes of disease gradients associated with citrus canker fluctuate over time due to disease-induced defoliation. However, most of these findings are the result of epidemiological research conducted in citrus nursery conditions and in the absence of the Asian leaf miner.

The Asian citrus leaf miner was identified in Florida in 1993 and in São Paulo in 1996 and quickly spread throughout both areas. The interaction between the leaf miner infestation and citrus canker was immediately apparent. In São Paulo, the number of foci increased 93 times between 1996 and 1999 (45 and 4180 foci, respectively); the number of diseased trees increased 85 times in the same period (3,512 and 299,856); in 1999, 1,737,545 diseased and 'exposed' trees were destroyed, compared with 30,394 in 1996 (an increase of 57 times). In urban Miami, the incidence of diseased trees increased rapidly in the last few years; in one study area, for example, incidence increased from a single infected dooryard tree to 1,731 infected trees within a two square mile area in 18 months; also, it was determined that dispersal can occur over distances far exceeding 125 ft.

In São Paulo, the temporal increase of diseased trees occurred concomitantly with a dramatic change in the spatial pattern of the epidemic, i.e., strongly aggregated patterns, typical of citrus canker from 1957 to 1995, gave way to less aggregated and even random patterns. Satellite foci located far away from main foci became very common. This change in the spatial pattern of diseased trees is thought to be an effect of the leaf miner, despite the fact that the miner is not a vector of the pathogen. Based on the analysis of 203 maps of infected groves (just prior eradication) from several regions of São Paulo between 1998 and 2000, it was proposed that in the presence of the leaf miner the dispersal function of the disease changes from a negative exponential model to an inverse power law model. This change, according to recent literature based on computer simulation, could explain the patterns seen today in São Paulo. Additionally, recent research on *X. axonopodis* pv. *citri* dispersal carried out in controlled conditions suggests that the dispersal distance is usually within a hundred meters but, if atmospheric humidity is near 100% and air turbulence is present, it may extend over a thousand meters.

In São Paulo, from 1999, the eradication policy has changed due to the new epidemiological conditions. Thus, groves with incidences higher than 0.5% are now completely eradicated. Those with incidences below 0.5% have all diseased and 'exposed' trees (in a radius of 30 m) destroyed. In Florida, the eradication policy has also recently changed. After a 18-month epidemiological study in urban Miami, the old 'rule of 125 ft' (all citrus trees within a 125 ft radius of a diseased tree are regarded as 'exposed', and destroyed) was changed in 1999 to the new 'rule of 1900 ft' (all citrus trees within a 1900 ft radius of a diseased tree are regarded as 'exposed', and destroyed). This study involved a complete and repeated GPS-based census of about 15,000 healthy and diseased dooryard citrus trees, with a combined study area of approximately 10 square miles.

More epidemiological work in field conditions is needed to bring about a better understanding of the citrus canker pathosystem, which now includes the Asian leaf miner in Florida and São Paulo. Epidemiological knowledge is indispensable to improve chances of total canker eradication as well as to define the best tactics for disease management in case of failure of eradication efforts. The overall objectives of epidemiological studies of citrus canker can be summarized as: (1) the identification and quantification of risk factors relating to temporal and spatial spread of the disease, (2) the formulation and evaluation of risk algorithms, and (3) the implementation of risk algorithms in decision-making relating both to short-term eradication and long-term regulatory policies.

Biological and Cultural Control Methods: A Moderated Discussion Session

[1] Rui P. Leite, Jr. and [2] Tim S. Schubert

[1] Instituto Agronômico do Paraná - IAPAR, 86001-970, Londrina-Paraná, BRASIL
[2] Florida Department of Agriculture, Division of Plant Industry, Gainesville, FL 32614, USA

Abstract

Main subject areas that will be discussed relative to cultural and biological control methods for citrus canker are:

Cultural
1) Pruning to control plant growth and remove infected tissues.
2) Use of windbreaks to interfere with the dispersion of inoculum by windblown rain
3) Defoliation tactics
4) Use of different rootstocks to alter scion growth characteristics
5) Nutrition modifications to alter scion susceptibility
6) Systemic acquired resistance / induced resistance

Biological
1) Bacteriophage
2) Antagonists
3) Basic canker ecology as it relates to biological control